

**CLAIMS AS FILED - OZ 51241**

1. A modified cytochrome P450 monooxygenase which, in comparison with the wild-type enzyme, shows an altered substrate profile in the terminal and/or subterminal enzymatic hydroxylation of aliphatic carboxylic acids, owing to site-specific mutagenesis of its substrate binding region.
2. A monooxygenase as claimed in claim 1, which is derived from cytochrome P450 monooxygenases of bacterial origin.
3. A monooxygenase as claimed in claim 2, which is derived from *Bacillus megaterium* cytochrome P450 monooxygenase BM-3 with an amino acid sequence in accordance with SEQ ID NO: 2, which has at least one functional mutation in one of the following amino acid sequence regions: 24-28, 45-51, 70-72, 73-82, 86-88, 172-224 und 352-356, with the proviso that, if the enzyme carries the mutation F87A, more than one of these regions is mutated.
4. A monooxygenase as claimed in claim 3, which comprises at least one functional mutation in the amino acid sequence regions 86-88 and 172-224.
5. A monooxygenase as claimed in claim 4, which comprises at least one of the following amino acid substitution patterns:
  - a) F87V;
  - b) F87A L188K;
  - c) F87V L188K;
  - d) F87A L188K A74G;
  - e) F87V L188K A74G;

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- f) F87A L188K A74G R47F;
- g) F87V L188K A74G R47F;
- h) F87A L188K A74G R47F V26T; or
- i) F87V L188K A74G R47F V26T;

and functional equivalents thereof.

6. A monooxygenase as claimed in claim 3, which comprises a single amino acid substitution from amongst the following:

- a) V26T,
- b) R47F,
- c) S72G,
- d) A74G,
- e) F87V,
- f) L188z, where Z is an amino acid selected from amongst K, R, W, Q, N, G, A and S, and
- g) M354T;

and functional equivalents thereof.

7. A nucleic acid sequence encoding a monooxygenase as claimed in claim 1 and the complementary nucleic acid sequence thereof.

8. An expression construct comprising, under the genetic control of regulatory nucleic acid sequences, an encoding sequence which encompasses a nucleic acid sequence as claimed in claim 7.

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9. A vector which encompasses at least one expression construct as claimed in claim 8.
10. A recombinant microorganism which has been transformed with at least one vector as claimed in claim 9.
11. A microorganism as claimed in claim 10, selected from amongst bacteria of the genus *Escherichia*.
12. A process for the enzymatic production of terminally or subterminally hydroxylated aliphatic carboxylic acids, which comprises
  - a1) culturing a recombinant microorganism which has been transformed with a vector which encompasses an expression construct comprising, under the genetic control of regulatory nucleic acid sequences, a sequence which encompasses a nucleic acid sequence encoding the monooxygenase of claim 1 in the presence of a culture medium which contains at least one hydroxylatable carboxylic acid or at least one hydroxylatable carboxylic acid derivative; or
  - a2) incubating a reaction medium containing at least one hydroxylatable carboxylic acid or at least one hydroxylatable carboxylic acid derivative with an enzyme as claimed in claim 1, and
  - b) isolating the resulting hydroxylated product from the medium.
13. A method as claimed in claim 12, wherein the hydroxylatable carboxylic acid is a C<sub>8</sub>-C<sub>30</sub> monocarboxylic acid or a derivative thereof.

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14. A method as claimed in claim 13, wherein the hydroxylatable carboxylic acid is a C<sub>8</sub>-C<sub>12</sub>-monocarboxylic acid or a derivative thereof and the monooxygenase used comprises at least one of the following amino acid substitution patterns:
- a) F87V;
  - b) F87A L188K;
  - c) F87V L188K;
  - d) F87A L188K A74G;
  - e) F87V L188K A74G;
  - f) F87A L188K A74G R47F;
  - g) F87V L188K A74G R47F;
  - h) F87A L188K A74G R47F V26T; or
  - i) F87V L188K A74G R47F V26T.
15. A method as claimed in claim 13, wherein the hydroxylatable carboxylic acid is a C<sub>12</sub>-C<sub>30</sub>-monocarboxylic acid or a derivative thereof and the monooxygenase employed is a mutant selected from amongst the single mutants F87A, F87V, V26T, S72G, A74G and M354T, and the multiple mutants F87A L188K A74G R47F; F87V L188K A74G R47F; F87A L188K A74G R47F V26T; or F87V L188K A74G R47F V26T.
16. (amended) A method as claimed in [any of claims] claim 12 [to 15], wherein the

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reaction is carried out in the presence of an electron donor or a reduction equivalent.

17. A method as claimed in claim 16, wherein the electron donor or the reduction equivalent is selected from amongst NADH, NADPH and zn/CO(III) sepulchrates.